HEXACHLOROBENZENE IN A SOUTHERN OCEAN FOOD WEB; CONTAMINANT ACCUMULATION & GLOBAL COMPARISONS

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Abstract
Further to a recent baseline survey of legacy and emerging persistent organic pollutant (POP) contamination in the Antarctic keystone species, Antarctic krill *(Euphausia superba*) from the eastern Antarctic sector[1], additional samples of phytoplankton and humpback whale (*Megaptera novaeangliae*) blubber, were analysed. Results corroborate previous findings that unlike the Arctic where PCBs and HCH dominate chemical profiles, chlorobenzenes and particularly hexachlorobenzene (HCB) dominate in the Southern Ocean food web[2]. Here we collate current findings and existing data from the Southern Ocean in order to draw critical comparisons of HCB contamination with Arctic and temperate northwest Atlantic trophic level counterparts.

Introduction
Hexachlorobenzene (HCB) and related chlorobenzenes are semivolatile, cyclic aromatic compounds with relatively high bioaccumulative potential (K_{OW} = 5.5) and long half-life in biota (~9 years).[3,4] HCB has been used in industry and agriculture since the 1930's, primarily as a fungicide however is also a by-product or impurity generated in the production of a large number of chlorinated compounds[4]. Primary emissions of HCB are estimated to have peaked in the 1970's[4] with a recent assessment of sources placing current emission rates in the range of 0.023-0.092 kt/year[5]. HCB is a known immunosuppressant and has been linked to reproductive and neurological impairment, endocrine disruption and non-mutagenic tumour promotion[3,6].

HCB was first detected in Antarctic krill and fish in 1979.[7] Since this time only ~25 articles have reported HCB in Antarctic biota and environmental matrices. Studies have primarily been restricted to the Antarctic Peninsula and the Ross Sea (~60°W and ~170°W respectively) with only a handful of studies of HCB contamination ever reported from the Australian Antarctic Territory (AAT), the largest of all the Antarctic territories (~44 -160°E).

In an effort to establish a comprehensive baseline for POP contamination and predator exposure in the eastern Antarctic sector, samples of adult Antarctic krill (*Euphausia superba*), the Antarctic keystone species, were collected from across a 50 degree latitudinal gradient and analysed for over 100 legacy and emerging persistent organic pollutants, including dioxins, PCBs, organochlorine pesticides and PBDEs. In addition we conducted opportunistic sampling and analysis of phytoplankton from the same region and humpback whale *(Megaptera novaeangliae*) blubber collected from a deceased adult animal located off the southeast Queensland coast. Our results corroborate previous findings which have indicated HCB to be the dominant organohalogen compound among POPs accumulating in the Southern Ocean food web.[2] Here we present our current findings and compare results with HCB distribution and concentrations from two diverse global regions.

Materials and Methods
Krill and phytoplankton samples were collected onboard the Australian Antarctic Division's flagship, the Aurora Australis, during the 2005/2006 Broke West krill survey of the Eastern Antarctic sector. Krill sampling stations were located at 12 sites between 30 and 80°E (Figure 1). Krill were collected by either target or regular trawls conducted using a RMT-1+8 net[8] in combination with hard codends to preserve the condition of the catch. Phytoplankton samples (n=4) were sieved continuously from the ship's clean seawater supply line whilst in the Southern Ocean.

Humpback whale blubber (n=1) was collected from a dead adult animal, presumed northward migrating, located off the southeast QLD coast in July 2006.

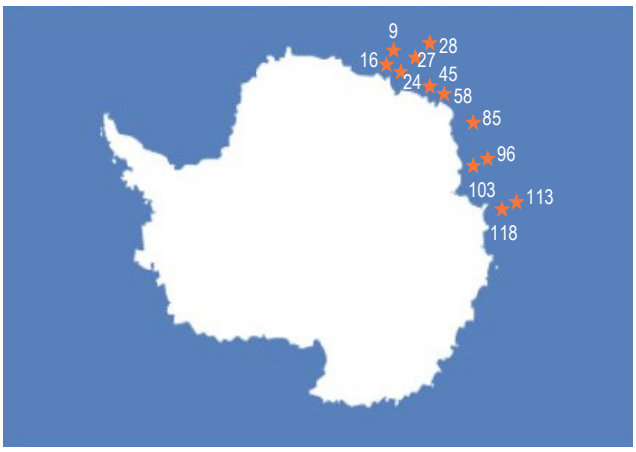


Figure 1 Krill sampling stations between 30 and 80° E

Sample preparation and chemical analysis was carried out according to standard operating procedures at the Norwegian National Institute for Air Research (NILU). The laboratory is accredited according to Norwegian standards for the analysis of chlorinated pesticides in biological material according to the requirements of NS-EN ISO/IEC 17025. Analytical procedures have been detailed elsewhere[9]. In brief, samples were homogenised with Na_2SO_4 and spiked with $^{13}C_{12}$-labelled extraction standard. HCB was extracted with an appropriate volume of cyclohexane:ethyl acetate (1:1) or cyclohexane:dichloromethane (1:1). The sample matrix was removed by multi-column chromatography using silica gel and activated charcoal. Samples were further cleaned by either HCL treatment (phytoplankton), gel permeation chromatography (blubber) or both (krill). A final treatment using sulphuric acid coated silica and alumina was performed on all samples prior to analysis. A method blank was incorporated every 5-7 samples. Extracts were analysed with GC/MS using a Hewlett Packard 5890II gas chromatograph coupled to a VG AutoSpec mass spectrometer.

Results & Discussion

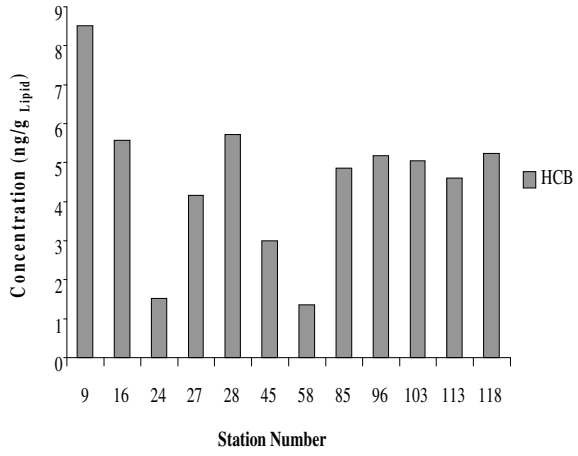
Table 1 summarises the results from the present and previous studies that have investigated HCB contamination in Antarctic and Arctic organisms. In addition we have selected the temperate and highly industrialised region of the northwest Atlantic for comparative purposes.

Table 1 Concentrations of HCB (present study in parentheses) (ng/g lipid) detected at three trophic levels in three global regions. If more than one reference has been cited, the mean concentration for all samples and studies is reported.

Taxa Region	Phytoplankton	Krill/herbivorous zooplankton	Mysticete Whales
Antarctic	*(5.7)* 4.1[10, present]	*(4.5)* 8.2[10-14, present]	*(91.0)* 122.3[2, 15, present]
Northwest Atlantic	Not reported	6.4[16]	132.8[17,18]
Arctic	23.0[19,20]	12.7[21-23]	132[24]

Our pilot investigation quantified HCB in 3 of 4 phytoplankton samples at an average concentration of 5.7 ng/g lipid. HCB has only once previously been reported at this trophic level in the Southern Ocean.[10] Chiuchiolo et al (2004) analysed summer phytoplankton and ice algae west of the Antarctic peninsula and found average HCB concentrations of 2.5 and 15.9 ng/g lipid respectively, reflecting the capacity of ice to act as a reservoir for persistent organic pollutants.[25] Phytoplankton from the Arctic Ocean and Barrow Strait were analysed for HCB by Hargrave et al. (1992)[19] and (2000).[20] These studies revealed concentrations of 25 and 21 ng/g lipid respectively. No published reports on HCB contamination at this trophic level could be found from the temperate Northwest Atlantic.

Both the present pilot study and all of the above cited studies omitted a record of the volume of seawater filtered in the collection of phytoplankton samples hence care should be taken in comparison of findings. Oceanic systems, particularly at high latitudes, are subject to seasonal cycles of productivity. These cycles in turn greatly influence the volume of particulate matter suspended at surface layers of the water column. Partitioning coefficients of hydrophobic chemicals will determine their distribution between the aquatic and particulate phase, however an increase in the total volume of suspended particulates will reduce the overall concentration of the contaminant associated with the particulate phase. In order to adequately interpret data-sets therefore, additional information regarding the filtered water volume is required.[26] Our present study season coincided with the height of the austral summer when phytoplankton biomass in the Southern Ocean is at its highest. Concentrations reported should therefore be interpreted as representing the lower range of seasonally fluctuating phytoplankton HCB concentrations in this region.



The results of accumulated HCB burdens in Antarctic krill are presented by sampling station in Figure 2. In general, the concentrations quantified in the current study (1.4-8.4, x=4.5 ng/g lipid) are comparable to those previously reported for the species (i.e. 3.8 - 13.3 ng lipid)[10-14]; Arctic krill and herbivorous zooplankton (i.e. 16[21], 19.7[23] and 2.4[22] ng/g lipid) and the levels reported in zooplankton sampled off the east coast of Newfoundland and Labrador (6.4 ng/g lipid).[16] Some variation is however evident within the Antarctic and Arctic cohorts where multiple reports are available. Assuming a diet of predominantly phytoplankton to be a constant for all species, variation may be attributed to a number of factors. Geographical locations as well as time of sampling are both among important variables to consider. The time of sampling will not only be reflected in the feeding habitat of zooplankton in polar environments (e.g. sea-ice edge or open ocean) but also the dominant life-history stage of the population captured (e.g. pre or post spawning adults).

Figure 2 HCB concentrations detected in Antarctic Krill (*Euphausia superba*) sampled at 12 sites across the eastern Antarctic sector

The average quantified concentration of HCB in inner and outer layers of humpback whale blubber in the present study was 91.4 ng/g lipid. No other studies have previously reported organochlorine contaminant burdens in Southern Ocean humpback whale populations which differ from their northern hemisphere counterparts in body size, the higher latitudes of their feeding grounds as well as diet, with northern hemisphere humpback whales supplementing a their krill diet with fish. Two publications however report HCB concentrations in Southern Ocean populations of the smaller mysticite whale species, the minke whale (*Balaenoptera acustorostrata*). Tanabe et al (1995)[15]and Aono et al (1997)[2] both report average concentrations of 127.4 ng/g lipid HCB in 59 and 97 male minke whales respectively.

A representative Arctic trophic level comparison is provided by the mysticite bowhead whale (*Balaena mysticetus*). Hoekstra et al (2002)[24] analysed blubber from 72 Bowhead whales harvested by native subsistence hunters in Arctic Alaska from 1997- 2000 and found an average HCB concentration of 132 ng/g lipid.

The Gulf of St. Lawrence is a major summer feeding ground for Atlantic migrating whale species. Metcalfe et al. (2004)[18] and Gauthier et al. (1997)[17] analysed blubber biopsies from adult mysticete whales summering in the Gulf (humpback whales (20)[17,18], blue whales (*Balaenoptera musculus*) (29)[17,18] and minke whales (21)[17]). The combined average concentrations of HCB reported in these studies was 132.8 ng/g lipid.

Results of the current study and reviewed literature demonstrate the highly dispersible nature of HCB and exemplify its capacity to bioaccummulate along food webs. Distribution between our 3 selected global regions does not show marked variability. The high volatility and moderate partitioning coefficients of HCB favours long atmospheric residence time and its potential for re-mobilisation. These properties have led Barber et al. (2005)[4] to hypothesise that HCB will reach a global equilibrium quickly compared with other POPs. The current study and review support this theory.

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